

Claims

1. A transgenic non-human animal, wherein said transgenic non-human animal comprises somatic or germline cells having incorporated into the genome of said cells a transgene that expresses an epitope-tagged TATA-box binding protein (TBP).

5

2. The transgenic non-human animal of claim 1, wherein said epitope-tagged TBP comprises two epitope-tags.

10

3. The transgenic non-human animal of claim 2, wherein said epitope-tagged TBP comprises human TBP (hTBP).

15

4. The transgenic non-human animal of claim 3, wherein said two epitope-tags comprise an HA-epitope and a His-epitope.

5.

15

5. The transgenic non-human animal of claim 4, wherein said epitope-tagged TBP comprises the amino acid sequence of SEQ ID NO. 16.

20

6. The transgenic non-human animal of claim 1, wherein the transgenic animal is a mouse.

25

7. A method of making said transgenic non-human animal of claim 1, comprising introducing a transgene encoding said epitope-tagged TBP into cells selected from the group consisting of:

(a) germline cells of said non-human animal;

(b) somatic cells of said non-human animal; and

(c) both germline cells and somatic cells of said non-human animal.

8. The method of claim 7, wherein said transgene is introduced into said cells by microinjecting said transgene into a zygote of said non-human animal.

9. The method of claim 7, wherein said transgene is introduced into said cells by transfecting a blastomere of said non-human animal with a vector comprising said transgene .

10. The method of claim 7, wherein said transgene is introduced into said cells by introducing said transgene into an embryonic stem cell of said non-human animal.

11. The transgenic non-human animal of claim 1, wherein said transgene comprises a first DNA sequence that encodes for TBP and a second DNA sequence that encodes for one or more epitope-tags.

12. The transgenic non-human animal of claim 11, wherein said first DNA sequence is a cDNA sequence.

13. The transgenic non-human animal of claim 12, wherein said cDNA sequence is the cDNA of human TBP (hTBP).

14. The transgenic non-human animal of claim 11, wherein said second DNA sequence encodes for two epitope-tags.

25 15. The transgenic non-human animal of claim 14, wherein said two epitope-tags comprise an HA-epitope and a His-epitope.

30 16. The transgenic non-human animal of claim 15, wherein said HA-epitope comprises an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO.1, SEQ ID NO. 2, SEQ ID NO. 3, and SEQ ID NO. 4.

17. The transgenic non-human animal of claim 1, wherein said transgene comprises the nucleotide sequence of SEQ ID NO. 13.

5 18. The transgenic non-human animal of claim 1, wherein said transgene further comprises an inducible or constitutive promoter.

10 19. The transgenic non-human animal of claim 18, wherein said constitutive promoter is the promoter of an elongation factor-1 alpha gene (EF) gene.

15 20. The transgenic non-human animal of claim 18, wherein said inducible promoter is the promoter of an metallothionein promoter (MT) gene.

20 21. The transgenic non-human animal of claim 19, wherein said transgene comprises the nucleotide sequence of SEQ ID NO. 14.

25 22. The transgenic non-human animal of claim 20, wherein said transgene comprises the nucleotide sequence of SEQ ID NO. 15.

30 23. A method of expressing an epitope-tagged TBP in a non-human transgenic animal, comprising introducing a transgene encoding said epitope-tagged TBP into cells selected from the group consisting of:
(a) germline cells of said non-human animal;
(b) somatic cells of said non-human animal; and
(c) both germline cells and somatic cells of said non-human animal, wherein said epitope tagged TBP is expressed in said non-human transgenic animal.

24. The method of claim 23, wherein said transgene further comprises a constitutive promoter.

25. The method of claim 23, wherein said transgene further comprises an inducible promoter and wherein said epitope-tagged TBP is expressed upon induction of said promoter.

5 26. A method for isolating a higher order transcription complex, comprising introducing a transgene encoding an epitope-tagged TBP into a non-human animal, expressing said epitope-tagged TBP in said animal, wherein said epitope-tagged TBP binds to one or more TBP-associated factors (TAFs) to create said higher order transcription complex, and isolating said higher order transcription complex from said animal.

10

27. The method of claim 26, wherein said higher order transcription complex further comprises a TAF-interacting factor bound to said one or more TAFs.

15 28. The method of claim 26, further comprising isolating said higher order transcription complex from more than one type of tissue or more than one cell type of said transgenic non-human animal.

20 29. The method of claim 26, further comprising isolating said higher order transcription complex from said transgenic non-human animal during two or more different developmental stages of said animal.

30. A method for isolating a TAF or a TAF-interacting factor, comprising introducing a transgene encoding an epitope-tagged TBP into a non-human animal, expressing said epitope-tagged TBP in said animal wherein said epitope-tagged TBP binds to said TAF or TAF-interacting factor to form a complex, isolating said complex, and separating said TAF or TAF-interacting factor from said epitope-tagged TBP.

25

30 31. The method of claim 30, further comprising determining the amino acid sequence of said TAF or TAF-interacting factor.

32. The method of claim 26, wherein said higher order transcription complex is isolated by contacting said complex with an affinity purification column comprising, as a solid phase, antibodies that bind to at least one of the epitopes of said epitope-tagged TBP component of said higher order transcription complex.

5

33. The method of claim 32, wherein at least one epitope of said epitope-tagged TBP is a His epitope and wherein said affinity purification column is a Ni²⁺.

10 34. The method of claim 32, wherein at least one epitope of said epitope-tagged TBP is an HA epitope and wherein said affinity purification column comprises, as a solid phase, anti-HA antibodies.

15 35. The method of claim 32, wherein at least one epitope of said epitope-tagged TBP comprises the amino acid sequence of SEQ ID NO. 17 and wherein said affinity purifying column comprises, as a solid phase, antibodies that bind to said epitope.

20 36. An antibody that binds to an epitope-tagged TBP, wherein said epitope comprises the amino acid sequence of SEQ ID NO. 17.

37. The transgenic non-human animal of claim 4, wherein said HA epitope comprises an amino acid sequence selected from the group consisting of SEQ ID NO.1, SEQ ID NO. 2, SEQ ID NO. 3 and SEQ ID NO. 4.